The Color Pattern of

Hermissenda crassicornis (Eschscholtz, 1831)

(Gastropoda : Opisthobranchia : Nudibranchia)

BY

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(9 Text figures)

INTRODUCTION

"The highly variable color of the living animals is generally transparent yellowish to bluish-grey, yellow-green or grass-green. The cerata may be translucent like the body, reddish with light blue, green, orange or white specks, probably cutaneous glands. . .The cnidosacs are transparent and separated from the rest of the cerata by a white, orange, yellow, purple or sometimes blue ring. On the outer side of the cerata runs a white line up to this ring. The smooth digestive diverticulum in the ceras is sand-colored, reddish to chocolate-brown, or black."

Reading this description of *Hermissenda crassicornis* (Eschscholtz, 1831) as given by Marcus, 1961, one realizes that it is difficult if not impossible to give a description of the "typical" color pattern in *Hermissenda,* since there is so much variation. However, a careful analysis of a large number of individuals shows that each individual's coloration is composed of the same basic elements according to the same basic rules.

In the first part of this study we shall describe the elements that make up the color pattern, and in the second part we shall consider some of the many color variants and the factors that may be responsible for color variation.

A similar and more extensive study has been done on a European aeolid, *Trinchesia coerulea* (Montagu) (Bürgin, 1961). It is worth noting that, although the two species differ in many details of color pattern, the basic principles of coloration were found to be the same in *Trinchesia* and in *Hermissenda.*

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To Professor Dr. A. Portmann, University of Basel (Switzerland), for reading the manuscript and helpful criticism; to Professor Dr. E. W. Fager, Scripps Institution of Oceanography, La Jolla (California), and his assistant, Miss Thea Schultze, for lending me optical instruments and laboratory equipment; to Mrs. Fay Wolfson, Mr. Wesley M. Farmer, and Mr. Clinton L. Collier for help with collecting specimens, and to Miss Esther Sandmeier, Zoological Institute of the University of Basel, for making the histological sections.

MATERIAL

Specimens of *Hermissenda crassicornis* were collected at the following locations:

1. La Jolla, rocky area near the "Cove"
   a) at low tide (—0.1 and lower) in tide pools, in May and June, specimens of up to 20 mm were abundant
   b) smaller specimens (up to 10 mm) were found in red algae collected from the rocks at low tide
2. San Diego, Point Loma, in tide pools, specimens up to 20 mm
3. San Diego, Dana Landing, specimens up to 40 mm in June (coll. Farmer & Collier)
4. Ensenada, Baja California, Mexico, in tide pools.

The largest specimens recorded in literature measured 55 mm (O'Donoghue, 1927). The largest specimens in our collection were 40 mm, the smallest ones 3.5 mm. Marcus (1961) gives a detailed account of the morphology and anatomy of the species, which will not be repeated here. However, the following points may be mentioned (Figure 1):

Marcus (*l. c.*) speaks of 11 groups of cerata, and a total of about 500 cerata. In our specimens there was always one distinct group in front of the pericardium, and a second one, equally distinct, behind the pericardium. Posterior to this the cerata of the different groups are so close together as to conceal their arrangement in

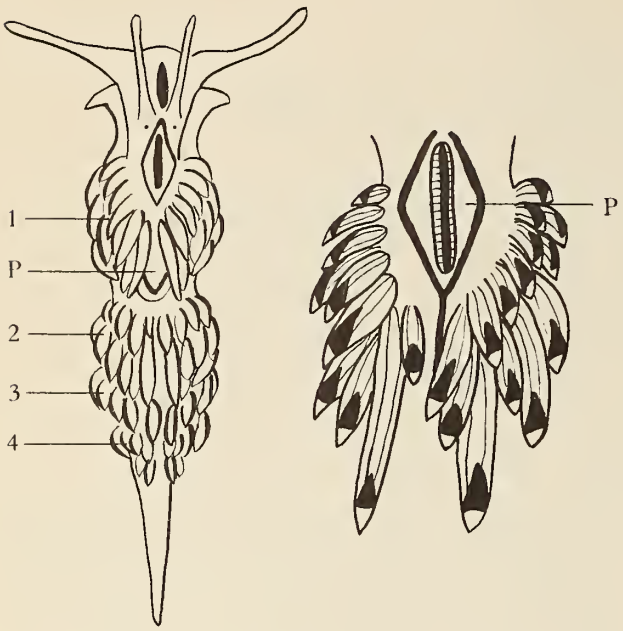

Figure 1: Left - living animal. specimen of 15 mm total length. Right - second group of cerata and pericardium with blue and orange pattern.
P - pericardium 1, 2, 3, 4 - designate groups of cerata

distinct groups. However, if all the cerata were removed, their arrangement in groups could be seen (6 in a specimen of 17 mm total length). The smallest specimen found (3.5 mm) had a total of some 24 cerata arranged in 3 groups. No attempt was made to ascertain the very complicated distribution pattern of the cerata within one group.

The longest cerata are found in the median region of the first two groups immediately in front of or behind the pericardium. It is not always the same pair that is the longest, nor are the longest cerata always corresponding ones on the right and on the left sides, respectively. Some of the median cerata (as well as others) are always in the process of regeneration, being much smaller, and usually hidden among the long cerata.

A. Components of the Color Pattern

Coloration of the ceras: The brown color of the cerata is due to the digestive gland shining through the partly transparent skin. The lower part of the long cerata and most of the smaller ones are dark brown; the upper portions of the longer cerata are lighter brown. Whereas the dark brown color is fairly uniform among all specimens, the lighter parts vary greatly in intensity and color shade, sometimes being almost yellow, in other individuals deep red-brown.

Further observation will show that the light and dark brown colors are due to two different kinds of cells in the digestive gland.

The skin of the cerata contains a varying amount of white, bluish- or greenish-white, light yellow or sky-blue granules. They may be scattered over a large area of the ceras like fine dust or form distinct lines, rings or patches, and they often display a high lustre.

There occurs yet another color, a deep orange, which is a true pigment dissolved in the cells of the epidermis. Where this diffuse orange pigment overlies the patterns formed by the white granules, the latter appear golden yellow.

Coloration of the body: The body pattern consists of blue lines running along the middle of the body and tail, forming two rhomboid patterns, one behind the rhinophores, a second one outlining the pericardium. White or bluish lines also run along the sides of the body between the groups of cerata. They all converge on the tail. Within the first, and sometimes within the second of the rhomboid patterns, and on the sides of the head there are very conspicuous orange markings. The orange patches on the back (though not those on the head) are composed of the same two color elements that were found on the cerata, yellow granules again forming a clear-cut line, the orange pigment being more diffuse, yet more intense in color.

In the laboratory the blue lines described above appear almost white or intensely blue, depending on the background. They show a metallic lustre and are composed of the same kind of granules as the white and blue designs on the cerata. If the animal is observed in one of its natural habitats, the tide pools with their lining of deep green eel grass, the lines on the body and tail appear in a very conspicuous shining green color.

Histological structure of the ceras —

Comparison of living tissues with sections: The ceras of *Hermissenda,* like aeolid cerata in general, has the following structure (Figure 2):

1. The epidermis consists of one layer of vacuolated cells with a thin cuticle and cilia. Between the ordinary epidermis cells gland cells are to be found (Figure 4. The cilia, except those at the tip of the ceras, are usually lost during fixation).
2. Next to the epidermis there is a layer of both circular and longitudinal muscles. The space between this outer layer of skin and muscles and the central digestive diverticulum is occupied by loose strands of connective tissue and filled with blood lacunae. It is this part of the ceras - the muscle layers, connective tissue and blood spaces - that give to the ceras of *Hermissenda* its extreme mobility and capability to contract and extend.

3. The central portion of the ceras is occupied by the diverticulum of the digestive gland with the cnidosac at its tip.

Although our study is confined to the cerata, it may be of general interest to note that in *Hermissenda* the cerata

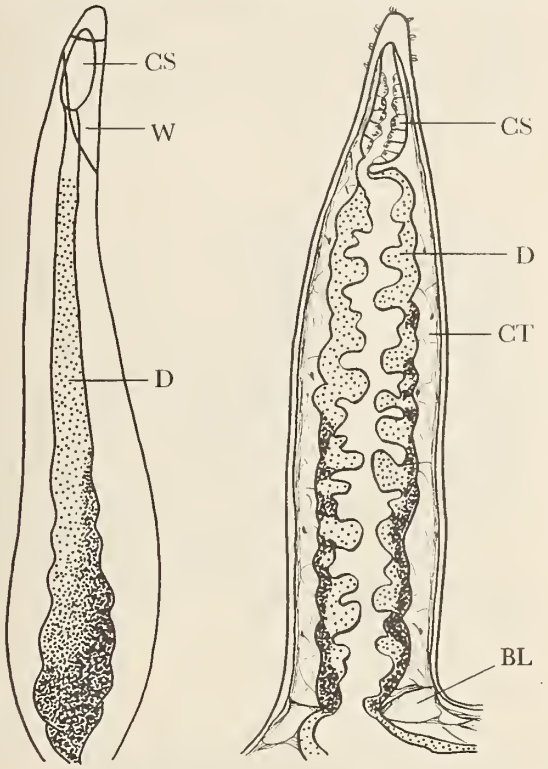

Figure 2: Left - side view of living ceras, showing dorso-ventral symmetry in pigmentation. Right - reconstruction of ceras from several histological sections (Bouin-Azan technique).
BL - blood lacunae
CS - cnidosac
CT - connective tissue
D - digestive diverticulum
W - white granules (sometimes light yellow)

alone contain the glandular tissue of the digestive gland. The ducts in the body are composed of flat, non-glandular epithelium.

In this paper histological details are given only as far as the color pattern is involved. For a more extensive description of the histology of an aeolid ceras the reader is referred to the paper on *Trinchesia* (Bürgin, 1961).

Skin pigments (Figures 3 and 4): Both the orange pigment and the white, blue or yellow granules are located in the epidermis. The orange pigment is more or less uniformly dissolved in the vacuoles of the epidermis cells. In cerata with little orange the pigment forms very light patches, leaving some parts entirely uncolored. In some specimens round bodies of deep orange are scattered in the pigmented area. They are located in the muscle layer underlying the epidermis.

The elements producing white, blue or yellow are located in the lower part of the epidermis cells as bodies of various shapes. Histological sections show that they occupy the enlarged basal portion of the cells, the nuclei having a distal position in relation to them. In these structural details *Hermissenda* is similar to *Trinchesia*.

Digestive diverticulum: The digestive epithelium in the ceras is not straight, but forms crypts and folds. The aspect presented by the microscopic preparation of a

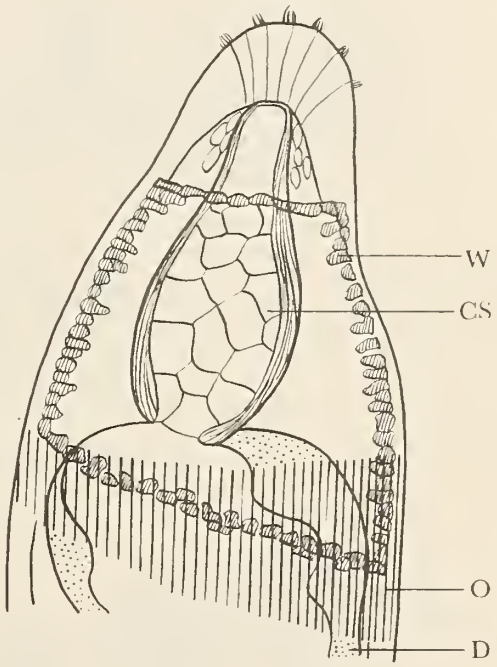

Figure 3: Tip of living ceras (side view), showing white granules and orange pigment (hatched). The white granules are shown only along the edge of the white area, in order to let cnidosac and digestive diverticulum shine through in the central area of the triangle.
CS - cnidosac
D - digestive diverticulum
O - orange pigment
W - white granules (sometimes light yellow)

living ceras is at first confusing. The following types of cells may be discerned in the digestive diverticulum (Figure 5):

a) Cells with thin-walled vacuoles, all of equal size, yellow, ochre, light green, colorless or orange

b) Cells with thin-walled vacuoles of varying size, colors as above except orange.

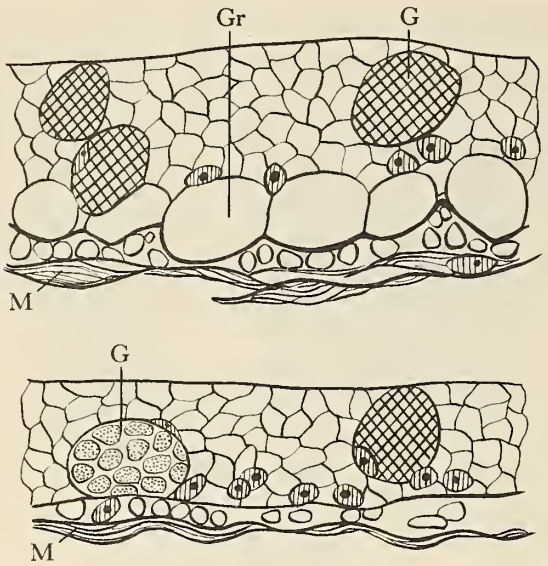

Figure 4: Epidermis of ceras. Histological section (Bouin-Azan). Top - Epidermis from region with white granules. Large holes are seen, where granules were in living tissue. Bottom - Epidermis from non-white area.

G - gland cell
Gr - granule or hole where granule was
M - muscles, circular and longitudinal

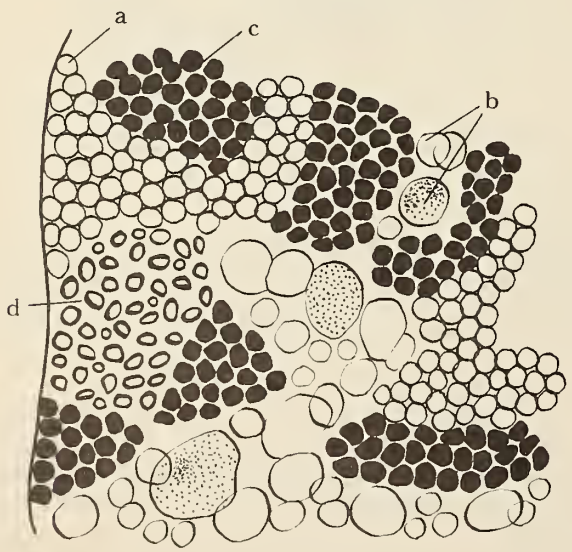

Figure 5: Part of digestive diverticulum of living ceras showing both digestive (a, b) and vacuole cells (c) in different aspects. (Letters refer to descriptions in text)

c) Cells with thick-walled polygonal vacuoles, densely packed, all of equal size, brown or red brown.
d) Cells with brown, purple or colorless granules of irregular shape, smaller than the vacuoles in (c).

In addition large vacuoles and round bodies, either yellow or colorless may be seen circulating in the lumen.

In a histological section fixed with Bouin's fluid and stained with either Hemalum-Benzopurpurine, Azan or Haematoxylin (Prenant) three different kinds of cells are found (Figure 6):

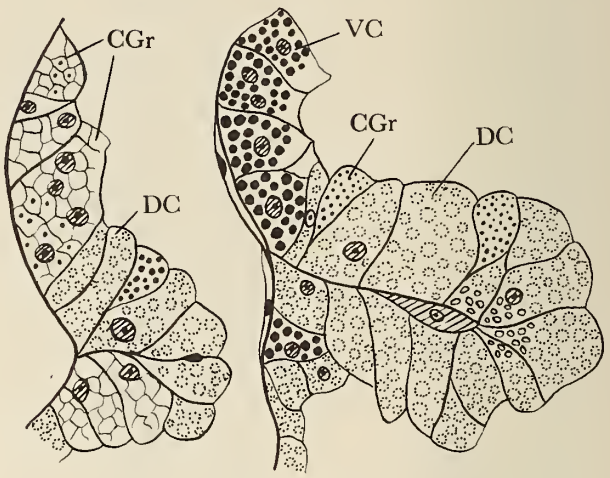

Figure 6: Histological sections of digestive diverticulum (Bouin-Azan). Left - apical part of ceras, no dark granules; in their place are cells with net of vacuoles and few small granules (CGr). Right - basal part of ceras, many vacuole cells with dark granules.

CGr - cell with granules
VC - vacuole cell
DC - digestive cell

1) Club-shaped cells with rounded bodies of equal or varying size, stained blue or orange in Azan, pink in Haemalum, green in Prenant (DC in Figure 6).
2) Cells of pyramidal shape with granules or vacuoles, all of the same size, which are not affected by the staining procedure but retain a dirty yellow or brown color of their own (VC in Figure 6).
3) Club-shaped or, less often, cylindrical or pyramidal cells with a net of vacuoles. Small granules may be found in the vacuoles. The plasma-net is stained orange in Azan; the granules are light brown (probably a color of their own) both in Azan and in Haemalum, but black in Prenant (CGr in Figure 6).

The club-shaped cells with round bodies of equal or varying size (1) are easily identified as the cells described

in the living tissue under (a) and (b). They will be called digestive cells. In many living animals digestive cells filled with vacuoles of an extremely intense orange are found at the base of the ceras. These vacuoles are stained deep red in Azan. All the other color differences often so conspicuous in the living tissue disappear upon fixation.

The pyramidal cells with brown granules (2) of the histological sections correspond to the cells with thick-walled vacuoles (c) of the living ceras. They will be called vacuole cells. Their distribution is characteristic: they are most numerous in the basal part of the ceras; toward the upper portion they become more and more scarce. There may be another cluster of them at the very tip of the digestive diverticulum, or they may be completely lacking there. These cells are more numerous in the dorsal part of the ceras than in the ventral one. They almost invariably occur at the periphery of the digestive diverticulum, in those parts of the crypts oriented towards the outside, the digestive cells occupying the more central parts along the folds.

The nature of the third kind of cells described under (3) in the living tissue and (c) in the histological sections is not clear. These cells are distributed along the entire height of the epithelium.

In *Trinchesia coerulea* two different kinds of cells had been found in the epithelium of the digestive gland, which correspond to the first two types described in *Hermissenda*. They were called "Verdauungszellen" (digestive cells) and "Körnerzellen" (cells with granules, corresponding to the vacuole cells)[1]. According to Graham (1939) the digestive epithelium of aeolids (*Aeolidina, Cratena*) consists of only one type of cell, the digestive cell, which carries out all the major functions of the digestive gland: production and secretion of enzymes, absorption of food, intracellular digestion, production and secretion of both fecal matter and (probably) true excretory products. In the paper on *Trinchesia* the extensive literature on this subject is discussed, and both from references in the literature and from personal observation the conclusion is drawn that the digestive cells in *Trinchesia* only "excrete" fecal matter, whereas the "Körnerzellen" produce true excretory products. These latter cells are considered to represent a cell type of their own.

In the present brief study on *Hermissenda* this question was not investigated any further; yet the general resemblance between the epithelium of *Trinchesia* and that of *Hermissenda* suggests that in *Hermissenda* too the digestive cells carry out all the functions of the digestive gland except the production of excretory substances, the latter being done by the vacuole cells.

The varying aspects of the third kind of cells may perhaps represent different stages in the metabolic cycle of either the digestive cells (production and secretion of enzymes?) or of the vacuole cells (excretion of vacuoles).

Our comparison shows that the digestive cells are responsible for the very variable component in the coloration of the digestive diverticulum. We shall see that their color is directly dependent on the intake of food. The vacuole cells, on the other hand, which impart to the ceras its dark color, retain their color even during long periods of fasting, and the dark brown color is far more constant among individuals feeding on various diets. This is in accordance with the assumption that the metabolic cycle of these cells is relatively independent from that of the digestive cells, and that the contents of the vacuole cells are a particular endproduct of metabolism.

Physical and chemical properties of color-producing structures —

In discussions concerning animal colors, a distinction is generally made between "pigment colors" and "structural colors." Pigment colors are due to a chemical substance (i. e. pigment) that can be extracted and analysed. and that retains its identity and more or less its color under varied conditions.

Structural colors, on the other hand, are due to a physical effect that is produced not by the molecular structure of a pigment, but by certain special structures within the animal tissue, and only under certain defined external conditions such as the incidence of light. These colors disappear or are altered as soon as the special structures or the external conditions are changed.

The orange color found in *Hermissenda* is clearly due to a pigment, for it remains unchanged, whether viewed in reflected or transmitted light, and it can be dissolved (and could be extracted) by organic solvents.

According to the above definition the white, blue and yellow colors in *Hermissenda* and other aeolids are a combination of pigment and structural effect: there is an identifiable substance present in bodies of various shapes, which can be dissolved by certain chemicals. But the specific color of these bodies and their striking metallic lustre are presented only in reflected light. If they are viewed in transmitted light, there is no iridescence, and they appear dull grey-brown instead. The iridescent white, blue and yellow of *Hermissenda* must therefore be due to a structural peculiarity of these bodies.

If these color-structures are viewed in reflected light at low magnifications (10 × 10), each element has a color of its own. Some of them show a metallic lustre, others do

[1] Cells similar to the third kind in *Hermissenda* were found in *Trinchesia*, but they were less numerous. They were then considered to be a variety of the Körnerzellen.

not. If the light source is moved, the distribution of iridescent and opaque elements is changed. The color that each element shows in transmitted light is roughly complementary to its color in reflected light, blue corresponding to yellow, bluish-green to copper red or purple, and vice versa. In transmitted light very often the margin of each body is yellow or ochre, and its centre either green or purple.

Further magnification of these color-structures (10 × 40 to 10 × 100) shows that they are vacuoles with an elastic wall, filled with small particles of oval shape (Figure 7). These small particles are colored yellow, green, blue or purple. Sometimes the particles in one vacuole are all of the same color, sometimes particles of different colors occur in one vacuole, such as yellow ones around the margin, red or green ones in the center.

The same general structure of these vacuoles was observed in *Trinchesia* and a number of other Mediterranean aeolids, and two species of *Glossodoris*. The size of the particles was then determined as follows:

Diameter about 1μ; Thickness 0.4 - 0.5μ

Particles of less than 1μ in diameter are often found, and, although more rarely, large ones having a diameter of 2 to 3μ. No measurements were made in *Hermissenda*, but the particles are of the same order of magnitude as in other aeolids.

Vacuoles which are blue or green in transmitted light usually contain larger particles less densely packed than those which have a red color in transmitted light.

In one instance the structure of blue-producing elements on the tail of *Hermissenda* could be seen more clearly than usual: around the margin of the vacuole thin platelets were arranged side by side like the spokes of a wheel. They appeared yellow. The center of the vacuole was red, and small round particles were indistinctly seen.

In the paper on *Trinchesia* the physical principles causing this structural effect are discussed in some detail, and the conclusion is drawn that the yellow and blue color and the metallic lustre of yellow, blue and white in *Trinchesia* are most probably due to interference phenomena.

Interference colors arise when light waves are reflected from the surfaces of thin multiple laminations, which are surrounded by material possessing a contrasting refractive index and whose thickness is of the order of magnitude of the lightwaves. In our case the oval particles about $\frac{1}{2}\mu$ in thickness enclosed in the vacuoles represent the thin laminations. Since these small structures are at the limit of resolution of an ordinary light microscope, it is difficult to obtain information on the exact way of color production. Yet the following observations may be worthy to recorded.

Certain relationships between the color effect and the structure of the vacuole were observed. The color effect produced depends partly on the arrangement and shape of the entire vacuoles and partly on the size of the particles within them. On some of the largest cerata, white of a diffuse, dusty appearance occurs. The color-structures in these areas are slender, widely branched bodies distributed loosely in the skin. Definite lines along the cerata, or the triangles on the tip of the ceras on the other hand are composed of vacuoles of a more compact shape lying close together (Figure 7).

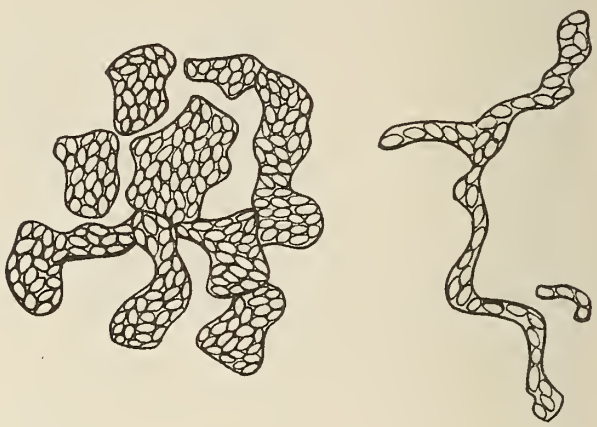

Figure 7: Vacuoles producing iridescent blue, white or yellow, from living ceras.
Left - part of yellow triangle, vacuoles of compact shape
Right - diffuse, dusty white, very slender vacuoles. Within the vacuoles particles producing interference phenomenon are seen.

On the tail of *Hermissenda* both white and blue lines lie side by side. The white is sometimes opaque, sometimes iridescent, the blue is always lustrous. In the opaque white lines vacuoles with very small particles, which are hardly distinguishable at a magnification of 10 × 100, are crowded very close together. They appear dirty brown in transmitted light, and no color is seen. White with a metallic lustre is produced by vacuoles with larger particles less tightly packed. The individual elements here have the typical colors mentioned above. According to Mason (1926-1927, quoted by Fox, 1953), who observed similar degrees of opaque and iridescent white in butterflies, opaque white is the result of diffuse reflection of light, and iridescence is due to interference.

If vacuoles of all colors are mixed randomly, as in some of the lines on the tail of *Hermissenda*, white is produced. In a blue region, such as the blue lines on the tail and body, and the blue patches on some cerata, most

elements are either plain blue (in reflected light) or green-blue, and yellow or red elements are extremely rare or lacking altogether.

In some specimens of *Hermissenda* yellow lines occur on the cerata below the range of the orange pigment. In these regions vacuoles golden yellow or copper-red in reflected light prevail, and green or blue elements are rare. It must be noted, however, that most of the yellow color seen superficially in *Hermissenda* is either due to the orange pigment combining with white producing structures, or to the effect of the underlying brown digestive diverticulum that gives the white pigment a yellow appearance. At the tip of the ceras, where there is neither orange pigment nor digestive gland, yellow was not found, but only white. In *Trinchesia coerulea,* on the other hand, structures such as those described in *Hermissenda,* produce a golden yellow color which is very intense even if the orange pigment is lacking.

Solubility: The solubility of the color elements in *Hermissenda* was tested (see below). The solubility of the orange pigment in chloroform and in alcohol suggests that it is a carotenoid. The white, yellow and blue color elements, being soluble in dilute acids and alkali, and insoluble in organic solvents, might belong to the group of purines and pterines. No difference in solubility between white and blue regions could be detected. Slight differences between the effect of HCl and NH_4OH on iridescence and dissolution of the vacuoles were observed, but the differences were not consistent. Sometimes the bodies would be dissolved more readily in NH_4OH, sometimes more rapidly in HCl. Table 1 shows that any treatment with chemicals, whether organic or inorganic, immediately alters the structures responsible for iridescence.

Ordinary fixation for histological purposes (Bouin, alcohol) leads to the destruction of both the orange and white or blue colors. The white structures can be preserved if fixing fluids free of acids, such as Helly's fluid, are used. The orange pigment always disappears because of the extensive treatment with alcohol.

B. Variation of the Color Pattern

Description of different variants: One of the most constant features in the pattern of *Hermissenda* is a white or very light yellow triangle at the tip of the ceras, overlying the cnidosac and the most distal part of the digestive diverticulum. The extreme tip of the ceras is always unpigmented. The lower part of this triangle appears golden yellow because of the orange pigment overlying it.

There may be more white, blue or yellow in the region proximal to the triangle. It is the arrangement and color of these color structures that vary most in the cerata of different individuals and even in cerata of a single specimen. There may be:

— a single straight line, or a broken line, white, blue, light yellow or greenish-yellow

Table 1

	Solubility		
	white, blue, yellow structures	orange dissolved in cells	orange bodies in muscle layer
Inorganic Solvents			
1% HCl	structure immediately altered, iridescence disappears, vacuoles dissolved after 10 to 30 minutes	unchanged	color changed, bodies remain
1% NH_4OH	as above	unchanged	unchanged
Organic Solvents			
Chloroform	structure immediately altered, iridescence disappears, vacuoles remain	dissolved	not dissolved within 30 min.
90% Isopropyl alcohol	structure immediately altered, iridescence disappears, white changed to pink! vacuoles remain	dissolved	not dissolved

— a large blue patch covering most of the dorsal side of the ceras
— several white or blue or partly white, blue, light yellow, and greenish patches of irregular shapes
— white granules scattered like fine dust, non-iridescent
— no white, blue or yellow at all.

In Figure 8 are shown a few examples of the various patterns found on the cerata of *Hermissenda*.

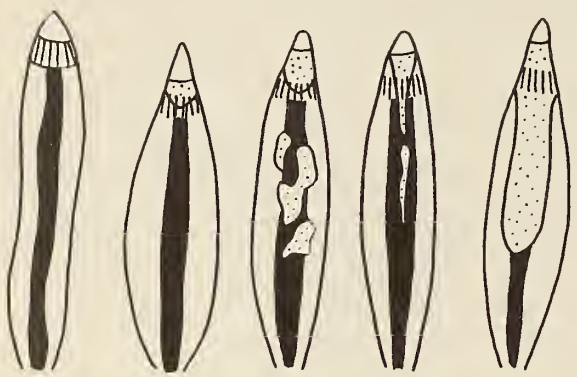

Figure 8: Largest cerata of five different specimens, showing some of many color patterns. Orange = hatched; white or blue = dotted

The intensity and extent of the orange pigment is also variable. The orange may be present only as a narrow ring in the upper zone of the ceras, overlying the region where the digestive diverticulum and the cnidosac meet, or it may extend more than half way down the ceras. In relatively few specimens is it completely lacking.

The digestive diverticulum is usually dark brown at its base. Sometimes the extreme tip is also darker than the middle zone. The color of the middle zone, which is due to the digestive cells and dependent on food will be described in a later section.

Pigment distribution: In the study on *Trinchesia* two general rules concerning the pigmentation of aeolid cerata were stated:

1. The visible, dorsal ("upper") part of the ceras is always more heavily pigmented and carries a more complete color pattern than the invisible, ventral ("lower") part of the ceras. The most conspicuous elements of the color pattern in *Hermissenda*, i. e. the yellow triangle and white or blue lines or patches are confined exclusively to the dorsal part of the ceras. The orange pigment most often extends farther down the ceras on the dorsal than on the ventral side. Even the vacuole cells of the digestive diverticulum, which are responsible for the dark brown color, are much more numerous in the dorsal half of the digestive diverticulum than in the ventral one.
2. The proportions of the color patterns are not the same in cerata of different sizes. Elements of the pattern such as rings, lines, triangles, patches, spots, are relatively larger on small lateral than on long median cerata (the cnidosacs also are proportionately larger in short lateral cerata).

In *Trinchesia* the golden yellow and blue rings could be measured fairly accurately. Since the color pattern in *Hermissenda* is less clear-cut, and the cerata are very mobile and contractile, no such measurements were made. But Figure 8 clearly shows that in *Hermissenda* the yellow triangle is relatively larger on small cerata. The number of vacuole cells too is relatively larger in small cerata. In lateral cerata the entire digestive diverticulum is dark brown, whereas in long median cerata only the basal part and sometimes the tip are dark brown.

Factors influencing coloration: The factors influencing coloration may be roughly stated as follows:

1. Age of specimen
2. Food or other environmental factors
3. Constant features in one individual.

1. Age: In some of the Mediterranean aeolids it is very obvious that as individuals grow they accumulate an increasing amount of those pigments, which are distributed evenly over a large area of the cerata or body, such as the violet in *Coryphella pedata,* the dark brown in *Facelina rubrovittata,* and to a lesser degree the orange in *Trinchesia coerulea,* whereas the relative amount of color-structures, such as the white, blue, or yellow, remains about the same.

It is true that our very small specimens of *Hermissenda* (3 - 5 mm) had little orange pigment, yet there does not seem to be a direct relationship between growth and the increase in orange pigment. Specimens of 10 to 20 mm may have a much deeper orange color than large ones of 30 to 40 mm.

2. Food: In order to study the influence of food on the color of cerata, feeding and regeneration experiments were carried out. Specimens of *Hermissenda* were fed with:

— Cerata or parts of body of other *Hermissenda* (being cannibalistic, the animals have to be kept separately!)
— Various species of hydroids
— *Anthopleura*
— Gonads of sea urchins

In one instance, where a deep red hydroid was used as food, the cerata of *Hermissenda* became distinctly red brown within one or two days. Otherwise a simple change of diet does not produce significant color changes in the cerata.

If normal healthy animals are kept without food for days or even months, the digestive diverticula in the cerata become extremely slender, and the upper light brown part eventually becomes almost transparent. The dark brown color at the base and in the small cerata, however, remains practically unchanged.

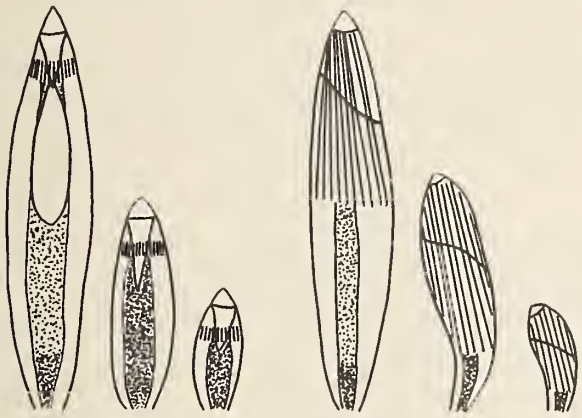

Figure 9: Sets of large, medium and small cerata of two different specimens, showing that color pattern is relatively larger on small cerata. Left - front view; Right - side view. Orange = hatched.

Regeneration: All the large cerata of a healthy animal are removed, and the animal is then kept in fresh sea water that is changed daily, for several days. Even without food the animal will usually regenerate its cerata within about a week or ten days to about one third of the original length. The regenerated cerata are transparent and have almost no color. If the animal is then given food, for example sea urchin eggs, the foodstuff is seen to enter and color the stomach and from there the digestive diverticula within a few hours.

It is the digestive cells of the digestive gland that take in the food particles, and these vary in color according to the food. The dependence of their color on food is a very direct one. The most extreme results were obtained with the orange gonads of sea urchins, and with hydroids, whose endoderm was red, producing yellow or red-brown digestive cells respectively. With the other diets, such as *Hermissenda*-parts, other hydroids or sea anemones the correlation was not as striking.

In contrast to the digestive cells, the contents of the vacuole cells are not influenced in the same degree by the nature of food. The dark elements of the ceras do not appear immediately after the fasting regenerating animal has been fed, but only two days later, and the color of the vacuoles is fairly constant irrespective of the food.

Other environmental factors that might affect the coloration of a specimen, such as water temperature or salinity, were not investigated in the present study.

3. Stability of individual differences: Regeneration experiments were carried out in order to determine whether the particular color pattern of an individual is a constant feature in that particular individual, and is reproduced during regeneration in the same way as it had been before.

All the longer cerata of 18 specimens were removed, the characteristics of their color pattern having been recorded. The animals were kept singly in aquaria and fed with cerata of other *Hermissenda* or sea urchin gonads (all specimens were given the same type of food at the same time).

The process of regeneration will briefly be described:
Small transparent humps are visible as early as the second day after operation.
On the fourth day tiny cerata with a light brown digestive diverticulum and a transparent cnidosac, but otherwise unpigmented, are present.
About the seventh day the first color structures appear, usually a light-yellow or greenish-white dot at the site of the triangle.
From the tenth day onward some orange pigment may be found.
After two weeks the typical color pattern begins to be recognizable. The distal white elements form a triangle, and there may be some white, yellow or blue basally to it. The cerata have now attained an average length of 2 mm, that is half or two thirds of their original length.

Of the 18 specimens in this particular experiment (some 20 others had been operated on for preliminary experiments to determine the general course of regeneration), 14 survived for two weeks or more after the operation. The longest survival was 30 days. With regard to the color pattern the following results were obtained:

The amount and arrangement of the orange pigment clearly is not fixed individually, but must depend on external factors. In the appearance or lack of blue or white, on the other hand, a certain tendency to produce the same individual pattern as before the operation can be recognized. No blue appeared in specimens which had not had it before the operation, and all those which had possessed a large amount of it originally deposited at least some blue in some of the regenerated cerata. It must be noted, however, that the distribution of blue on the many cerata of an individual is completely random; it is not the same ceras that is marked with blue before and after regeneration, and the shapes of the deposits also vary.

Table 2

	original pattern, before operation	regenerated pattern 14 to 30 days after operation
Blue Color		
6 animals	conspicuous blue on some cerata	blue present on some cerata
4 animals	some blue	some color-structures on cerata basally to triangle, blue or white
4 animals	no blue at all	no blue
Orange		
10 animals	orange present	orange present, but arrangement on cerata different
2 animals	orange absent	orange present
1 animal	very much orange	almost no orange
1 animal	orange	in the same way as before

General remarks on regeneration: The following observations made during these regeneration experiments may be worth recording.

Specimens of *Hermissenda* can survive without food for over a month in the aquarium; they even regenerate their cerata up to a certain point. During this time the tail is resorbed progressively until it is no more than a very short stump. Even if the animals are fed regularly, their tails become shorter during the regeneration process, and the animals decrease in body length as much as 25% (reducing from 20 mm to 15 mm, for example).

In *Trinchesia* we observed that in cerata regenerated without the animal being fed, the structures producing the yellow and blue in the skin appeared, but the fat-soluble orange pigment was lacking. This was considered to be further evidence that the orange pigment is a carotenoid derived from food. In *Hermissenda* the orange pigment does appear in cerata regenerated without food; but the animal seems to have considerable reserves of carotenoids in the body, in the gonads and also in the orange stripes on the back and on the head.

If all the cerata of a *Hermissenda* are removed - they can be picked off easily with watchmaker's forceps - some tiny ones which are too small to be grasped with the forceps, always remain, mostly on the sides, but a few on the back. The latter must have appeared after the loss of a ceras, but were prevented from regenerating as long as all the other ones were present. We observed in fact that if only two median cerata are removed, they never grow longer than one quarter of their original length during the period of one month. If, however, all the other cerata are lost suddenly, these remaining "buds" grow very rapidly during the first days following operation, until they are almost twice as long. Lateral cerata do not grow out of proportion, but the median ones can be recognized as the longest and most developed ones even as long as three weeks after the operation.

A typical example of regeneration is given here.

Length of animal	12 mm
Longest cerata	2 mm
fourth day after operation:	
regenerating cerata	0.5 mm
left-over cerata on back	1.2 mm
left-over cerata on sides	about 1 mm
19th day after operation:	
Length of animal	8 mm
most regenerating cerata	1 - 1.5 mm
left-over cerata on back	2 mm
left-over cerata on sides	about 1 mm

It seems that the cerata which are prevented from regenerating to full length serve as a reserve among the full-grown, functioning ones and take over only if an accident occurs.

SUMMARY

The color pattern of *Hermissenda crassicornis* consists of the following elements:

1. Epidermis
   a) Fat-soluble orange pigment, probably a carotenoid, dissolved in the cells of the epidermis.

b) "Color structures" or granules, soluble in dilute acid and alkali, located in the lower part of the epidermis cells, that produce white, blue or yellow, sometimes iridescent color.

2. Digestive diverticulum
   a) Digestive cells, giving the ceras a light brown, ochre or red brown color, depending on food.
   b) Vacuole cells, dark brown in color, which are less directly dependent on food.

The structure of the white, blue or yellow producing elements is described in some detail. It is assumed that iridescence is due to an interference phenomenon caused by minute particles contained in vacuoles.

Some of the color patterns found most often in cerata of *Hermissenda* are described. The extreme variability of coloration observed in this species is due on the one hand to differences in the amount and distribution of the four basic color elements, and on the other hand to actual color differences of the digestive cells, whose color depends on food.

Feeding and regeneration experiments throw some light on the question as to which features of the color pattern are influenced by external factors, and which are constant in an individual.

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